PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 1515SG112SC	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/SG2004/000093	International filing date *(day/month/year)* 14 April 2004	Priority date *(day/month/year)* 14 April 2003
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] C12Q 1/68, 1/66		
Applicant TEMASEK LIFE SCIENCES LABORATORY et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      - [X] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:

[X]	Box No. I	Basis of the report
[ ]	Box No. II	Priority
[X]	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
[ ]	Box No. IV	Lack of unity of invention
[X]	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
[ ]	Box No. VI	Certain documents cited
[ ]	Box No. VII	Certain defects in the international application
[X]	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
14 February 2005	28 July 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **ALISTAIR BESTOW** Telephone No. (02) 6283 2450

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International application No.
PCT/SG2004/000093

Box No. I Basis of the report

1. With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:

   ☐ international search (under Rules 12.3 and 23.1 (b))

   ☐ publication of the international application (under Rule 12.4)

   ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the elements of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

   ☐ the international application as originally filed/furnished

   ☒ the description:

   pages **1 - 17** as originally filed/furnished
   pages* received by this Authority on with the letter of
   pages* received by this Authority on with the letter of

   ☒ the claims:

   pages as originally filed/furnished
   pages* as amended (together with any statement) under Article 19
   pages* **18 - 20** received by this Authority on **17 February 2005** with the letter of **14 February 2005.**
   pages* received by this Authority on with the letter of

   ☒ the drawings:

   pages **1/3 – 3/3** as originally filed/furnished
   pages* received by this Authority on with the letter of
   pages* received by this Authority on with the letter of

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing (*specify*):
   ☐ any table(s) related to the sequence listing (*specify*):

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing (*specify*):
   ☐ any table(s) related to the sequence listing (*specify*):

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos: **13 – 19 (partially)**

because:

☐ the said international application, or the said claims Nos.
relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos.
are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos.
are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claim Nos. **13 – 19 (partially).**

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
- ☐ has not been furnished
- ☐ does not comply with the standard

the computer readable form
- ☐ has not been furnished
- ☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

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Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1 – 12, 20, 21 (completely), 16 - 19 (partially)	YES
	Claims	13 - 15	NO
Inventive step (IS)	Claims		YES
	Claims	1 - 21	NO
Industrial applicability (IA)	Claims	1 – 12, 20, 21 (completely), 16 - 19 (partially)	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The claimed invention relates to a method of identifying the presence of a transgene of a genetically modified organism, by adding a primer which hybridises to the transgene, subjecting the sample and primer to polymerase reaction, and enzymatic detection of the pyrophosphate which is released during the polymerase reaction thereby signalling the presence of the transgene.

Citations

D1 WO, A, 1998/013523

D2 WO, A, 1998/028440

D3 US, A, 4971903

D4 EP, A, 630974

D5 WO, A2, 2002/064830

D6 WO, A, 2000/040750

D7 WO, A, 1998/066653

D8 WO, A, 1992/016654

D9 Analytical Biochemistry (1996) 242:84-9

D10 Analytical Biochemistry (1993) 208:171-5

D11 Genome Research (2000) 10:1249-58

D12 Science (1998) 281:363-5

D13 Proceedings of the Symposium on Bioluminescence and Chemiluminescence,12th, Cambridge, United Kingdom, Apr. 5-9, 2002 (2002), 395-398.

D14 Analytical Biochemistry (1997) 244:367-73.

D15 Detection' Analytical Biochemistry (2001) 288:28-38.

Novelty (N) and Inventive Step (IS)

D1 discloses a method of identifying a base at a target position in a nucleic acid by adding a primer, which hybridises to the target. This is followed by a polymerase reaction in which pyrophosphate is released. The enzymatic detection of the pyrophosphate provides a real time indication of the incorporation of the deoxynucleotide, and thereby is indicative of the presence of the target nucleic acid. (see claim 1)

(continued in Supplemental Box)

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 13 - 19 are not fully supported by the description. It is agreed that the claimed kits are directed to kits 'for use' in the invention. However, the claimed kits, when they are framed in terms of being 'for use', are not limited to the particular use provided in the method of the invention. The only support for the kit is when it is being used in the method of the invention. The phrase '...for use in a method...' means only that the claimed kit needs to be capable of being used in the method, and not that it is being so-used. There is inadequate support for a claim to the kit when it is not being used in the method of the invention.

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

   [X] a sequence listing

   [X] table(s) related to the sequence listing

   b. format of material

   [X] in written format

   [ ] in computer readable form

   c. time of filing/furnishing

   [X] contained in the international application as filed

   [ ] filed together with the international application in computer readable form

   [ ] furnished subsequently to this Authority for the purposes of search and/or examination

   [ ] received by this Authority as an amendment* on

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:V

The citation expresses a preference for the use of the luciferase system to detect the pyrophosphate and the use of solid supports. (see Example 1) While the citation may not describe the method as being specifically useful for the detection of the presence of a genetically modified organism, such a purpose would be obvious to the skilled addressee faced with the problem of detection genetically modified organisms. D1 does not limit the types of targets which could be detected by the method of the invention, and indeed the skilled addressee, wishing to detect a particular target, whether located in a genetically modified organism or otherwise would be aware that the teachings of D1 could be used to detect any target, by selecting the appropriate primer. The skilled addressee would still use the teaching of D1 in the same manner as the presently claimed invention to detect a genetically modified organism. The nature of the target does not affect or limit the methods to which the organisms carrying the target are treated. D1 is directed to detecting a base in a target position, and this includes a target in a genetically modified organism. Therefore claims 1 – 12, 20 and 21 lack an inventive step.

D1 also discloses kits for use in the process (see claims 10 – 11) which comprise the same components as those the subject of claims 13 - 15. Presently, the claimed kits are not limited to being used in the method of the invention. It is evident that the kits disclosed in D1 could be used for the same purpose specified in claims 13, 14 and 15. Therefore these claims lack novelty and inventive step. Remaining claims 16 – 19 to kits, which specify particular detection means and primers are not inventive, as these features are variations which would be obvious to the skilled addressee. The applicant does not suggest that the choice of primers used in these claims provides any particularly surprising result, these having been selected merely to demonstrate the method of the invention.

D2 discloses a method and a kit which is very similar to that of D1. This also compromises the novelty of claims 13, 14 and 15 and the inventiveness of the remaining claims, for similar reasons as provided above.D3 discloses a method of determining the nucleic acid sequence of a template nucleic acid, located on a support having bound thereto a primer, in which the polymerisation occurs, and recovering therefrom the pyrophosphate generated during the process. The pyrophosphate is then detected using luciferase, which is indicative of the presence of the target DNA. While D3 does not specify the identification of a transgene in a genetically modified organism, the method claims 1 – 12 and 20 – 21 lack an inventive step for similar reasons as provided above. A kit of components as is claimed in claims 13 - 19 would be obvious to the skilled addressee with D3 on hand, and therefore these claims lack an inventive step for similar reasons as provided above.

D4 discloses a method of detecting a target nucleic acid comprising amplifying a target nucleic acid sequence, and in the process of so doing, generate inorganic orthophosphates which are detected by means of a colourimetric signal, thereby indicating the presence of the target. (see Example and claim 1). While D4 does not specify the identification of a transgene in a genetically modified organism, the method claims 1 – 12 and 20 – 21 lack an inventive step for similar reasons as provided above. A kit of components as is claimed in claims 13 - 19 would be obvious to the skilled addressee with D4 on hand, and therefore these claims lack an inventive step for similar reasons as provided above.

D5 discloses a method for determining the extent of a processive nucleic acid polymerase reaction producing pyrophosphate, by detecting the pyrophosphate by use of luciferase (see claim 1). The examples note the use of primers and the polymerase reaction to generate the pyrophosphate, and it is implied that the generation of the pyrophosphate denotes the detection of the primer. (see Example 2) While D5 does not specify the identification of a transgene in a genetically modified organism, the method claims 1 – 12 and 20 – 21 lack an inventive step for similar reasons as provided above. A kit of components as is claimed in claims 13 - 19 would be obvious to the skilled addressee with D5 on hand, and therefore these claims lack an inventive step for similar reasons as provided above.

(continued in Supplemental Box)

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: V

D6 discloses a method of determining a nucleotide base in a nucleic acid sample by incubating the nucleic acid with a primer and a polymerase, whereby any pyrophosphate released (and detected) is indicative of the presence of the target. (see p. 2 line 32 - p. 4 line 2, Example 1 and claims 1 - 7) While D6 does not specify the identification of a transgene in a genetically modified organism, the method claims 1 – 12 and 20 – 21 lack an inventive step for similar reasons as provided above.

D7 discloses methods of detecting the presence of a specific nucleic acid in a sample, by introducing a primer which is complimentary to a specific nucleic acid sequence, extending the primer using a polymerase, thereby generating a pyrophosphate which is detected. Detection of the pyrophosphate is indicative of the presence of the specific nucleic acid in the sample. (see p. 7 line 14 - p. 9 line 24, Examples and claims 1, 23 - 25) While D7 does not specify the identification of a transgene in a genetically modified organism, the method claims 1 – 12 and 20 – 21 lack an inventive step for similar reasons as provided above. A kit of components as is claimed in claims 13 - 19 would be obvious to the skilled addressee with D7 on hand, and therefore these claims lack an inventive step for similar reasons as provided above.

D8 discloses a process and a kit which encompass similar processes to those noted in D7, thereby compromising the novelty and inventiveness of the same claims noted above.

D9 discloses real-time sequencing , whereby nucleotides added to an immobilised template hybridised to a primer during a polymerase reaction. The pyrophosphate generated during the reaction is detected by a luciferase reaction, thereby signalling the presence of the target. (see Abstract and Figure 1.) While D9 does not specify the identification of a transgene in a genetically modified organism, the method claims 1 – 12 and 20 – 21 lack an inventive step for similar reasons as provided above. A kit of components as is claimed in claims 13 - 19 would be obvious to the skilled addressee with D9 on hand, and therefore these claims lack an inventive step for similar reasons as provided above.

D10 discloses a similar process to that of D9, and therefore the method claims 1 – 12 and 20 – 21 lack an inventive step for similar reasons as provided above. The preparation of kits comprising the elements noted in claims 13 - 19 would be obvious to a skilled addressee in the light of D10.

D11 - D15 disclose sequencing and detecting methods involving a primer followed by polymerase reaction and the generation and detection of pyrophosphate, in the same fashion depicted in claims 1 – 12 and 20 – 21. While D11 – D15 do not specify the identification of a transgene in a genetically modified organism, the method claims 1 – 12 and 20 – 21 lack an inventive step for similar reasons as provided above. A kit of components as is claimed in claims 13 - 19 would be obvious to the skilled addressee with any one of D11 – D15 on hand, and therefore these claims lack an inventive step for similar reasons as provided above.

Industrial Applicability (IA)

The matter of claims 1 – 12, 20, 21 (completely) and claims 13 – 19 (partially) appears to be industrially applicable.

CLAIMS

1. A method of identifying the presence of a transgene of a genetically modified organism in a sample wherein the nucleic acid of the transgene is replicated and detected as the release of pyrophosphate (PPi), the method comprising :

adding an oligonucleotide primer to the sample which hybridizes to the transgene;

subjecting the sample nucleic acid and primer to a polymerase reaction in the presence of a mixture of deoxynucleotides required for replication of the transgene whereby the deoxynucleotides are incorporated and release PPi proportional to the length of the DNA extension product; and

detecting any release of PPi enzymatically;

wherein release of PPi indicates the presence of the transgene.

2. The method of claim 1, wherein the transgene is replicated in a reaction selected from the group consisting of a polymerase extension reaction, a polymerase chain reaction (PCR), a ligase chain reaction (LCR), a rolling circle replication reaction (RCR) and a nucleic acid sequence based amplification reaction (NASBA).

3. The method of claim 2 wherein the transgene is replicated in a polymerase chain reaction.

4. The method of claim 1, wherein the release of PPi is detected by means of a Luciferase-Luciferin-based reaction.

5. The method of claim 1, wherein PPi release is detected using ATP sulfurylase and luciferase.

6. The method of claim 1, wherein the PPi detection enzymes are

included in the polymerase reaction step and the polymerase reaction and PPi release detection steps are performed substantially simultaneously.

7. The method of claim 1, further comprising adding a dATP analogue which is capable of acting as a substrate for a polymerase, but incapable of acting as a substrate for a PPi detection enzyme.

8. The method of claim 7, wherein the dATP analogue is deoxyadenosine. alpha. thiotriphosphate.

9. The method of claim 1, wherein the sample DNA or oligonucleotide primer is immobilized or provided with means for attachment to a solid support.

10. The method of claim 1, for use with a multiplicity of sample DNA sequences, wherein said DNA sequences are arranged in assay format on a solid surface.

11. The method of claim 1, wherein said nucleic acid sample is from a plant.

12. The method of claim 11 wherein the plant is a food source.

13. A kit for detecting the presence of a transgene of a genetically modified organism in a sample as defined in claim 1, comprising:

a polymerase;

an enzyme detection means for identifying PPi release;

deoxynucleotides, or optionally deoxynucleotide analogues, optionally including, in place of dATP, a dATP analogue which is capable of acting as a substrate for a polymerase but incapable of acting as a substrate for a PPi-detection enzyme; and

optionally a transgene specific primer which hybridizes to the transgenic DNA and is recognized as a primer by a polymerase, wherein the polymerase replicates the transgenic DNA.

14. The kit of claim 13, wherein the detection enzyme means comprises luciferin and luciferase.

15. The kit of claim 14, wherein the detection enzyme means comprises ATP sulfurylase and luciferase.

16. The kit of claim 13, wherein the transgene specific primer hybridizes to a transgene that provides herbicide resistance.

17. The kit of claim 16, wherein the transgene specific primer hybridizes to a transgene that provides resistance to the herbicides selected from glyphosate and glufosinate.

18. The kit of claim 13, wherein the transgene specific primer hybridizes to a transgene that provides insect resistance.

19. The kit of claim 13, wherein the transgene specific primer is selected from SEQ ID NOS. 1 to 29.

20. The method of claim 1, wherein the oligonucleotide primer is selected from SEQ ID NOS. 1-29.

21. A method of detecting a transgene of a genetically modified organism in a sample that may contain nucleic acid from the genetically modified organism wherein the nucleic acid of the transgene is replicated and detected as release of pyrophosphate, the method comprising:

adding an oligonucleotide primer selected from SEQ ID NOS. 1 to 29 to the sample;

subjecting the sample to a polymerase reaction; and

detecting the release of PPi;

wherein the release of PPi indicates the presence of a transgene in the sample.